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Intracellular Analysis With an Immobilized-Enzyme Glucose Electrode Having 2-μm Diameter and Subsecond Response Times

by

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INTRACELLULAR ANALYSIS WITH AN IMMOBILIZED-ENZYME GLUCOSE ELECTRODE HAVING 2-μm DIAMETER AND SUBSECOND RESPONSE TIMES

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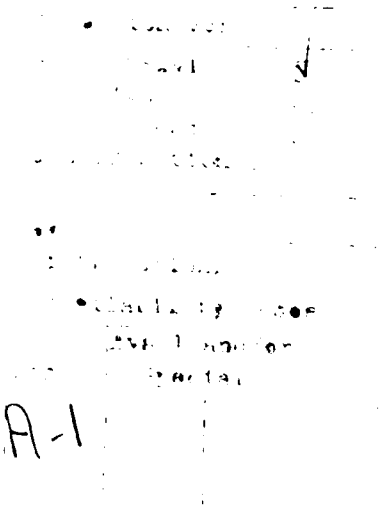

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ABSTRACT

Glucose oxidase has been immobilized on carbon ring electrodes having total tip diameters as small as 2 μm. This procedure has been used to construct glucose microsensors that are reasonably stable and have response times of hundreds of milliseconds. The principle of operation is based on amperometric sensing of hydrogen peroxide as a product of glucose oxidation by the enzyme. The amperometric response is relatively insensitive to oxygen levels. Transient changes in cytoplasmic concentration of glucose have been monitored in the large dopamine cell of the pond snail, Planorbis corneus.

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The search for small, rapid, and selective glucose sensors has resulted in a large repertoir of different electrode strategies (1). Platinized microelectrodes have recently been used with immobilized glucose oxidase to detect glucose with response times of only a few seconds (2). Pantano et al., (3) recently reported an enzyme-modified microelectrode constructed by linking horse radish peroxidase via a biotin/avidin/biotin tether to carbon fiber electrodes with 8-μm diameter defining the electroactive surface area an a slightly larger total structural diameter. These electrodes have response times on the order of 300 ms. In this communication, we present a simple procedure to construct enzyme-modified electrodes with total tip diameters as small as 2-μm using platinized carbon ring electrodes.

Carbon ring electrodes were constructed as described previously by Kim et al.(4). The general procedure for immobilizing glucose oxidase on the electrode was similar to that described by Ikariyama et al.(5). Carbon ring electrodes were platinized by reduction of 10 mM hexachloroplatinate in the presence of lead acetate for 3 min. The resulting porous platinum-coated electrodes were then oxidized in phcsphate buffer (0.5 M, pH 7.0) at 1.1 V vs a sodium saturated calomel

reference electrode (SSCE) for 15 min, oxidized in glucose oxidase (100 mg/mL) for 15 min, immersed in bovine serum albumin (5 % by weight), and finally a gluteraldehyde solution (1 %) to coat the glucose oxidase with a thin film of albumin and to complete the microsensor.

The microsensor response derives from the amperometric detection of hydrogen peroxide which is a product of glucose oxidation by the enzyme in the presence of oxygen. The response time of the electrode was determined using flow injection analysis with a stainless steel valve and trap injection system and a 68-μm i.d. silica capillary. Electrodes coated with glucose oxidase in this manner were checked for stability by observing the signal obtained in static solutions of 3 and 0.5 mM glucose for 20 hours. The losses in response over that period of time were found to be 27 % and 23 %, respectively.

Figure 1 shows the fastest response time obtained with a 2-μm total tip diameter glucose sensor. The rise time (6) is 270 ms and the fall time is 210 ms. The average response times for glucose microsensors having tip diameters less than 3 μm are 460 +/- 190 ms and 430 +/- 220 for rise and fall time, respectively (n= 4). Interestingly, response time of these electrodes is observed to increase linearly with electrode tip diameter. Over the range from 2 to 10 μm diameter the plot of response time vs diameter has a slope of 0.194 s/μm, a correlation coefficient of 0.9022, and an intercept of 0.022 s.

The selectivity of these microsensors was checked in static amperometric experiments by independent injections of enough

fructose, galactose, malutose, and KCl (control) to change the solution concentration by 0.1 M. No detectable response was observed. This strongly suggests that the response observed in solutions of glucose is due to oxidation of that substance via glucose oxidase and not due to direct oxidation at the electrode surface.

An important consideration in the use of hydrogen peroxide producing enzyme reactions for amperometric detection is the effect of oxygen concentration on the rate of glucose oxidation via the enzyme. Amperometric detection in a stationary electrochemical cell was used to determine this relationship. Figure 2 shows a plot of the average amperometric signal obtained with glucose microsensors placed in 0.5 mM glucose solution at varied oxygen concentrations. As the concentration of oxygen is reduced from one atmosphere to zero, the amperometric response is relatively unaffected until the oxygen concentration is below 0.24 mM. Similar data have been obtained (not shown) for solutions of 50 μM glucose and no variation is observed until oxygen concentrations as low as 50 μM are reached (3% variation in response at this concentration). This suggests that this type of sensor can be used to monitor glucose in the submillimolar range for experiments where the oxygen level is subject to limited variation, including in vivo analysis.

The development of ultrasmall sensors should eventually make it possible to monitor glucose in the cytoplasm of single functioning cells. Thus, with future developments it might be possible to monitor respiration in single cells of heterogeneous

organs (ie. the brain). Another area of interest is monitoring glucose regulation in single cell (ie. liver cells or adipocytes) cytoplasm following administration of cell affectors such as insulin, glucagon, or adrenalin. To determine the applicability of the glucose microsensors developed here they have been placed into single large dopamine cells of the pond snail, Planorbis corneus and used to measure intentional manipulations of cytoplasmic glucose. Dissection, manipulation, and intracellular voltammetry of neurotransmitters have all been described previously for this cell (7). Figure 3 shows the intracellular response observed following injections of glucose into the same cell in which a glucose microsensor has been placed. Control experiments have been carried out. No observable change in the amperometric current is observed when either 1) a glucose microsensor is used to monitor injections of phosphate buffer without glucose present or 2) a naked platinized electrode is used intracellularly to monitor cytoplasmic injections of glucose. With the glucose microsensor described, injection of glucose is monitored as a small, but rapid increase in cytoplasmic levels followed by a more gradual decline which is likely to represent cellular metabolism and storage of glucose under these conditions. Based on in vitro calibrations, the change in cytoplasmic glucose observed in this experiment corresponds to 0.8 mM and the entire response to a transient injection of glucose into the cytoplasm is finished in 2 min.

Despite the bulk-coated enzyme principle by which they are constructed, the glucose-oxidase electrodes used here have

response times similar to those reported by Pantano et al.(3) where horse radish peroxidase was covelently attached to the surface of the electrode. It is reasonable to assume that the smaller size of the electrodes reported here offsets the advantages of close localization of the enzyme to the electrode surface gained by a covalent link. Combination of covalent bonding of the enzyme via the biotin/avidin/ biotin link described previously (3) with our smaller electrodes (8) might provide response times approaching or less than the 100 ms mark.

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(6) Response times were evaluated as time for the signal to change from 10% to 90% of full response for rise times and from 90% to 10% for fall times.

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(8) The total tip diameters of these electrodes range from 2 to 10 μm; however, the thickness of the electroactive ring has been estimated by scanning electron microscopy to be in the range from 50 to 150 nm (Saraceno, R. A.; Ewing, A. G. J. Electroanal. Chem. **1988**, 257, 83-93).

FIGURE LEGENDS

Figure 1. Time course of the amperometric signal observed following a flow injection pulse of 0.5 mM glucose (FIA flowrate, 0.3 uL/min; electrode potential, 0.6 V vs SSCE).

Figure 2. Typical response of a glucose microsensor as a function of oxygen partial pressure. The glucose concentration was 0.5 mM in 0.5 M phosphate buffer at pH 7.0. Oxygen pressure was varied between zero and one atmosphere of oxygen partial pressure. Responses have been normalized to the signal at ambient atmospheric concentration of oxygen.

Figure 3. Amperometric current monitored at a 2-μm glucose electrode placed in the cytoplasm of the large dopamine neuron of the pond snail. This neuron has a diameter of approximately 200 μm and a volume of 4 to 6 nL. The arrows indicate 2 consecutive injections (2 pL) of 3 M glucose into the cytoplasm. Injections were carried out with an Eppendorf pressure-based microinjector system and a glass micropipette having approximately 1-μm tip diameter. Injection volumes were approximated by injection of water into oil and measuring the volume optically.

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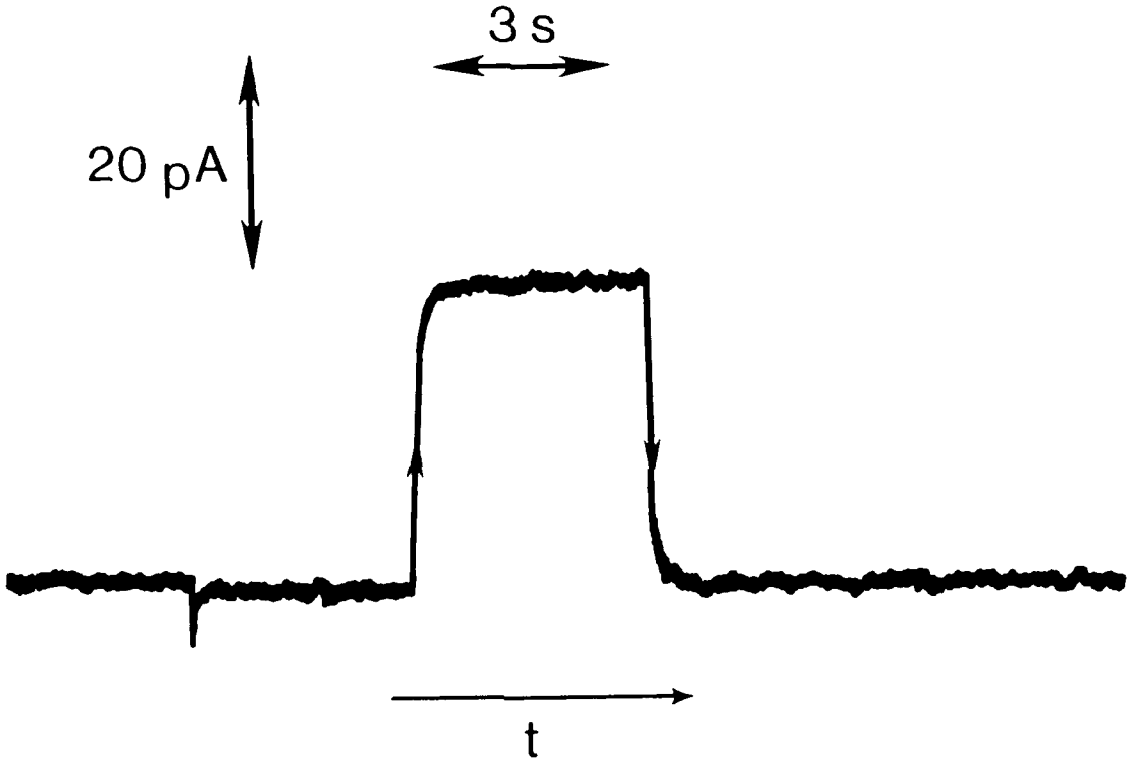
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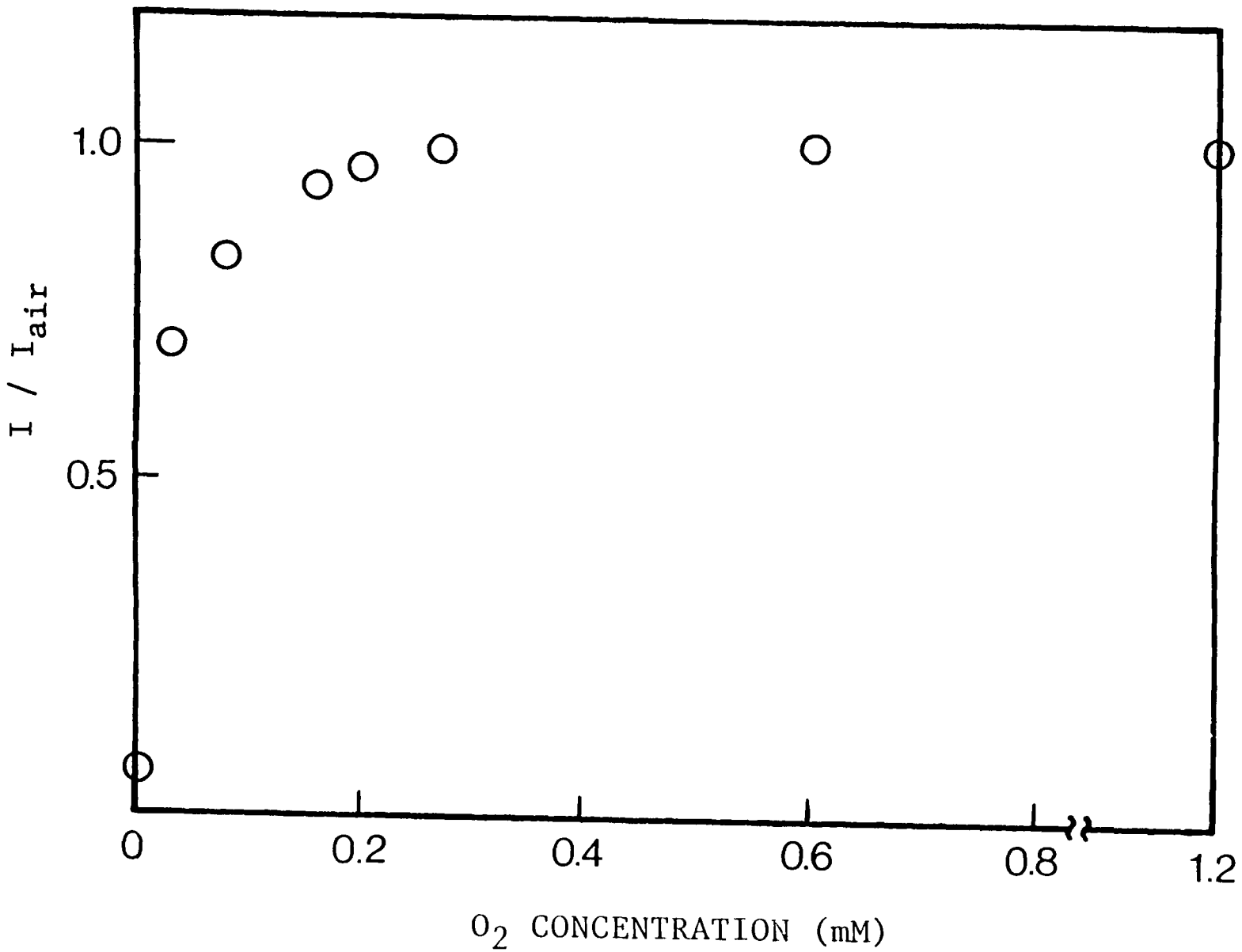
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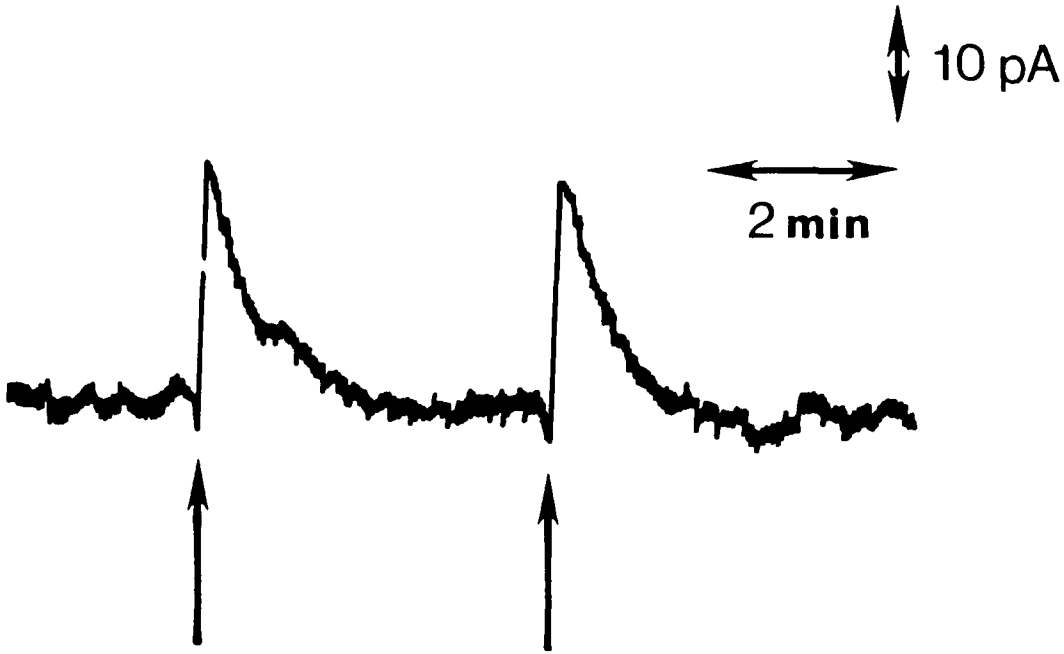
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